Clonal *Santalum album* growth, oil content and composition on different hosts and at different locations

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Abstract

The growth of clonal *Santalum album* on different hosts was assessed over 14 years at three different locations in Western Australia (Kununurra, Carnarvon and Geraldton). In trial one, two clones (K7 and LS) were grown on *Melaleuca* and *Azadirachta indica* as hosts at the three localities. Sandalwood growth was good at Kununurra and Carnarvon (increment of diameter at BH 0.8cm pa.), but poor at Geraldton. Heartwood was first detected in year 10 at Carnarvon. No heartwood formed at Geraldton up to year 14. Oil concentration in heartwood was highest at Kununurra (4.4% for the base of clone K7 with 65% santalols), and composition was similar to that published for juvenile trees growing in India. In the tallest trees, heartwood was present to 4m height in trunks, with only small differences between oil concentration and composition at different heights in the trunk.

In trial two, in which one clone (R32) was compared when grown at Kununurra with each of *Acacia mangium*, *Cassia siamea* or *Dalbergia sissoo* as a host, growth was best on *D. sissoo* and oil concentration highest using *A. mangium* as a host.

There was a range in growth and oil concentration within clones and improved silvicultural techniques are required if plantations are to benefit from the more uniform product theoretically possible from clonal material.

Keywords: *Santalum album*, oil content, oil composition, clones

Introduction

Santalum album L. (Indian sandalwood) an evergreen tree growing to 18 m, is prized for its aromatic heartwood. Although use of sandalwood from natural stands can be traced back 2,300 years in India (Srinivasan *et al.* 1992), there is some evidence it was introduced from Timor (Brand 1993, Fox *et al.* 1995). *S. album* grows in forests on the Indian peninsula, highland regions of eastern Indonesia and in Australia in scattered locations along the northern coast. It is cultivated in plantations in India, Indonesia, Australia and elsewhere (Radomiljac *et al.* 1998). World sources of sandalwood are declining rapidly (McKinnell 1993; Radomiljac *et al.* 1998) with significant harvest of plantation-grown timber yet to become available to the market. In India, poaching, adverse environmental conditions and spike disease are depleting sandalwood reserves while in Indonesia reserves are threatened by fire, grazing, and regulations that disadvantage the owners of the land on which sandalwood grows.

Seedlings of sandalwood are variable in growth rate, disease resistance, the age at which heartwood is initiated, and the content and quality of the oil (Srinivasan *et al.* 1992). The age at which trees initiate heartwood varies markedly with genotype and habitat. For example, Haffner (1993) reported that trees growing in West Timor ranged in age from 14–46 years for initiation of heartwood, while in India, heartwood initiation is generally expected within 10–13 years (Rai 1990). Also, the oil concentration and composition changes as trees mature. Young trees (about 10 years old, diameter >16 cm) have 0.2–2.0% oil, 60–85% santalols and higher santalyl acetate and santalenes than mature trees (30–50 years old, diameter ~32cm, 2.8–5.6% oil, ~90% santalols) (Srinivasan *et al.* 1992). It is the high level of the sesquiterpene alcohols α- and β-santalol in mature trees of *S. album* that is responsible for aroma and quality (Anonis 1998). In mature wood there is usually 50–60% α-santalol and 20–30% β-santalol with the latter contributing most to the typical sandalwood odour (Brunke and Hammerschmidt 1988).

In *S. album*, oil concentration is highest in roots and the butt of the tree, then decreases in the trunk and branches. The percentage of santalols decreases only slightly from the roots to the branches (Jayappa *et al.* 1981; Shankaranarayana & Pathasarathi 1987). In contrast, in *S. spicatum* there is a sharp change in oil composition at the root/shoot junction with α-and β-santalol both dropping markedly in concentration from the root to the trunk, and the concentrations of epi-α-bisabolol and t,t-farnesol increasing sharply (Piggott *et al.* 1997; Moretta 2001; Moretta *et al.* 2001).

Trees grown in different geographic areas are known to vary in oil content and composition, but it is difficult to disentangle genotypic differences and attribute specific differences to particular environmental conditions

(Jayappa *et al.* 1981). However, it has been suggested that trees which have higher rainfall and grow faster have a lower proportion of heartwood than those that grow more slowly due to water shortage. However hard data, particularly for plants given different management regimes on the same site, appear to be lacking (Applegate *et al.* 1990; Kealley 1991; Loneragan 1990).

Therefore, cloning superior trees appears a powerful tool to increase the productivity of plantations. Clones of superior trees were shown to have a faster growth rate and greater heartwood formation than seedlings, and after seven years, the selected clones showed measurements of girth and heartwood formation comparable to 50 year old trees (Lakshmi Sita 1991).

This paper reports on the effect of different hosts, and growth in different environments on oil content and composition of *S. album*.

Materials and Methods

Sources of plant material

Sandalwood clones

Clone R32 was raised from a four-year-old tree, grown from seed from Tangali, India, collected by Peter Richmond. (In 1990, it was one of the best trees on the CALM Arboretum in Kununurra, which was established in 1986.) Clone K7 was raised from a seedling from a tree grown at Kununurra in trials planted by Ian Richmond and Jen McComb. The provenance was Royalapad, India, and the tree was selected for good growth at year three in the Kununurra trial. Clone LS was from a tree grown in Lesmurdie (WA) from unselected seed.

Host plants

Plants of the temporary hosts *Sesbania formosa* or *Vigna unguiculata*, and of final hosts *Cassia siamea, Acacia mangium and Dalbergia sissoo*, were raised from seed. Later plantings of infill hosts were also raised from seed for planting in 1997 and 1988 at Carnarvon (*Cathormion umbellatum* a woody legume native to the Kununurra area) and at Geraldton (*Acacia acuminata* (jam)).

A clonal line of *Azaderachta indica* (neem) was selected from shoot cultures of seedlings grown in vitro. *Melaleuca quinqueuervia* was from cuttings of a single genotype and was purchased from Zanthorrea Nursery. *M. leucadeudra* was grown from cuttings in Kununurra.

Tissue culture protocols

Stem callus was induced from nodal sections of *S. album* trees cultured on solid Murashige & Skoog (MS) (1962) medium with 2.26 μM 2, 4-dichlorophenoxyacetic acid (2,4-D). Kinetin (2.23 μM) and indole acetic acid (5.71μM) were used in MS medium for callus proliferation and embryo induction. Embryogenic callus was transferred to medium with gibberellic acid (GA_3) (2.89 μM) for embryo maturation. Fully formed embryos were transferred to White's medium (Singh & Krikorian 1981) lacking plant growth regulators. Plantlets with both root and shoot apices growing were transferred to filter paper bridges dipping into liquid White's medium without sugar or growth regulators. All solid media contained 20 g L^{-1} sucrose, 2.5 g L^{-1} Gelrite and 2 g L^{-1} agar and had a pH of 5.7. Cultures were grown in polycarbonate tubs 7.5 X 7 cm and incubated at 25° C under constant light (30–40 $\mu mol.m^{-2}.s^{-1}$).

A. indica shoot multiplication was on MS with 2.5μM BAP, and rooting on 1/2 MS with 5μM IBA. Well grown rooted plants were potted and grown on in a glasshouse at Murdoch University. When sandalwood plants were 100 mm tall they were provided with an *Alternanthera* species as a pot host. Sandalwood was planted in the field when six months out of tissue culture (except for the replanting of Kununurra) and hosts were of a similar age.

Trial designs and assessments

There were two experiments. The first examined the effect of environment, and clones were planted at Geraldton, Carnarvon and Kununurra. The climate, soil and planting years at each site are described in Table 1. The second trial was at Kununurra and examined the effect of different hosts.

Table 1

Location, climate and soil at the three trial sites for sandalwood in Western Australia.

Location	Lat/Long	Mean rainfall (mm)/ irrigation	Soil type	Mean summer and winter daily maximum and minimum (lowest minimum, highest maximum) (°C)	Year planted
Frank Wise Research Station Kununurra	128° 44'E 15° 46'S	816 & flood irrigated	Cununurra clay	25.4–35.5 (47.8) 16.2–30.9 (7.9)	1991 replanted 1993
Dept of Agriculture Research Station Carnarvon	113° 66'E 24° 88'S	250 & drip irrigated	Sand to sandy loam, Gascoyne association light series	23.2–35.5 (49.2) 10.7–23.4 (1.1)	1991
40k N of Geraldton	114° 70'E 28° 78'S	425 & drip irrigated up to 3 times a week in years 1–5 then infrequent	Sandy clay	17.5–31.0 (46.0) 9.6–20.3 (2.0)	1991

Trial 1: The effect of environment: Trials of clones of S. album at Geraldton, Carnarvon and Kununurra

The two clonal lines of *S. album* were planted on similar final hosts at Kununurra, Carnarvon and Geraldton. In Kununurra, a temporary host (*Sesbania formosa*), was planted 1m from the sandalwood, and in Carnarvon *Vigna unguiculata*. No temporary host was used in Geraldton. Hosts and sandalwood were planted at the same time. The pigeon pea plants died within 12 months and the *Sesbania* within three years and are not considered further. At Carnarvon, it became evident over time that the sandalwood were also parasitising a roadside verge row of *Casuarina cunninghamiana*, as adjacent to the sandalwood trial the *Casuarina* heights were significantly reduced. At Geraldton, the tallest plants were 10 m from two mature native *Acacia acuminata*.

The permanent hosts were *Azadirachta indica* (syn. *Melia azadirachta*) neem, and *Melaleuca*. *M. quinquenervia* was used at Geraldton and Carnarvon and *M. leucadendra* at Kununurra. At Carnarvon and Geraldton, sandalwood and hosts were planted in the same row at 2 m spacing in the sequence neem, *Melaleuca quinquinerva*, sandalwood, *Melaleuca*, neem, sandalwood *etc*. At Kununurra, the sandalwood were planted on mounds, with plants 2 m apart in rows, with 2.5 m between (initially with the *Sesbania* 1m from each sandalwood), with the alternate rows being hosts in the row sequence *Melaleuca leucadendron*, sandalwood, neem, neem, *Melaleuca*, sandalwood *etc*. At Geraldton, after death of the neem an attempt was made to infill hosts by planting *Acacia acuminata* in 1997 and on the death of these plants this was repeated in 1998. These trees grew to 1–2m and are not considered further. At Carnarvon, *Calliormion umbellatum* was used to infill gaps. These had little impact: only 2 plants (12.5%) survived to 2005 reaching a mean height of 2.1m, and are not considered further.

At Carnarvon and Geraldton, there were four replicates of five plants of sandalwood clones K7 and LS. At Kununurra, there were three replicates of 10 plants of sandalwood clone K7.

Trial 2: The effect of host species: Comparison of sandalwood growth on three hosts at Kununurra

Clone R32 was planted on three final hosts *Acacia mangium*, *Cassia siamea* or *Dalbergia sissoo* in 1993. The field layout was as above with sandalwood provided with *Sesbania* as a temporary host. There were five rows of five sandalwood, alternating with rows of one of the three hosts and each host area was surrounded by a double row of the same host. Hosts and sandalwood were planted at the same time.

Initial, larger trials including all the sandalwood clones were planted at Kununurra in 1991 but were destroyed by corellas. Additional plants kept in pots for 18 months were used to set up the smaller trials outlined above in 1993.

Assessment of survival, growth and oil presence

Survival, height and trunk diameter were monitored at intervals. From year six onwards some of the larger sandalwood trees at Carnarvon and Geraldton were assessed for the presence of heartwood by drilling a 3 mm diameter hole at the base of the tree and testing the burned wood in the drill bit for the distinctive sandalwood odour.

Harvesting and oil analysis

In 2005, all remaining trees were harvested from the Kununurra plots and half of the trees from the Carnarvon plot. No trees were harvested at Geraldton. The trunk of each sandalwood was cut at 100 mm from the ground, then the soil around the tree was excavated and the remaining trunk and roots pulled from the ground. From each tree, a basal disk or "biscuit" of 30–50 mm in thickness was cut at ground level. In the comparison of clone R32 on different host species, from five of the tallest trees on each host, sample wood disks were cut both at the base level and at 1.5m height. In the comparisons of different clones at the same site, and of the same clone growing at different locations, for the K7, trees at Kununurra and for K7 and LS at Carnarvon, 4–5 trees were harvested at the base and at 1.5m height. A further analysis of the change in oil composition with position in the tree was carried out using one LS ramet and two K7 ramets from Carnarvon and two K7 ramets from Kununurra. From these, biscuits were cut at 20 cm intervals (to 1.6m) then 40cm intervals from the base to the top of the heartwood and from the base to the depth of roots where heartwood ceased. Tree diameter and percentage of area that was heartwood were assessed from the biscuits.

From each biscuit containing heartwood, a central section of 10 mm by 30 mm was marked. Biscuits without heartwood were not included. These sections were then cut out of the biscuits using a bandsaw and roughly powdered using a hand-held grinder. The ground samples were then processed and analyzed for oil concentration and composition, using a gas chromatography flame ionization detector (GC-FID) and a mass-selective detector (GC-MS), at Australian Botanical Products (ABP), Hallam, Victoria. The methods used by APB to extract the sandalwood oil and analyze the oil were similar to the methods described in Brand *et al.* (2007).

In summarising the data, values of zero for oil concentration (%) were included in the means of % oil concentration, but the values for α-and β-santalol were only included in the analysis when oil concentration was above 0.1%

Results

Trials of clones of *S. album* LS and K7 at Geraldton, Carnarvon and Kununurra

Survival and growth of the hosts at the three locations

At Geraldton, all neem died except for one plant that survived for 7 years but only reached 1m in height (Fig. 1). At Geraldton, neem was winter deciduous, while it was evergreen at the other locations. Melaleucas at Geraldton had 100% survival, reaching 5.7m after 10 years and thereafter showing no increase in height (Fig. 2). The neem grew well at Carnarvon with the best trees reaching a height of 9–10m, but over time they were damaged or killed by the sandalwood, reducing the final mean height to 4.2m and survival to 60% (Fig. 1).

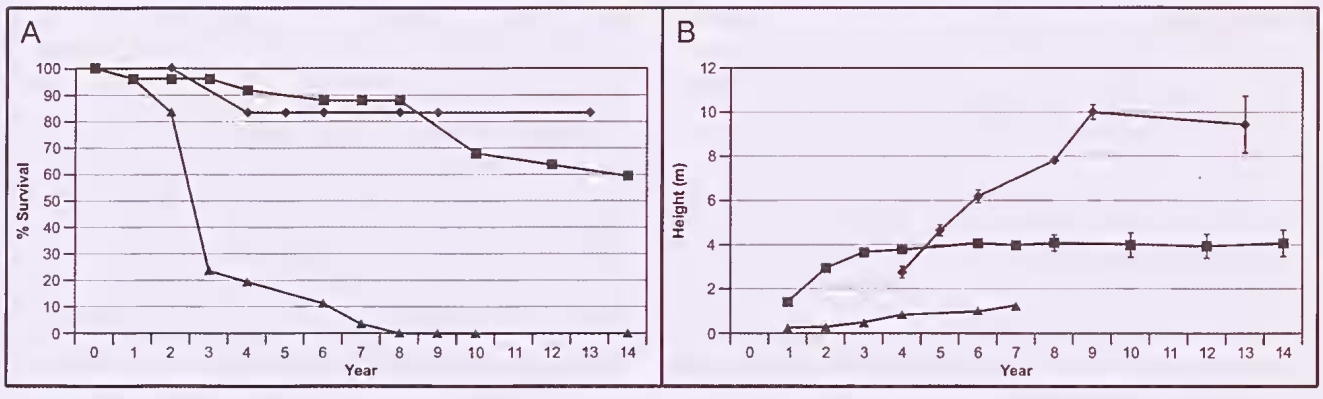

Figure 1. Survival (A) and mean growth (B) of neem at Geraldton ▲, Carnarvon ■ and Kununurra ◆. Bars on height data indicate standard errors.

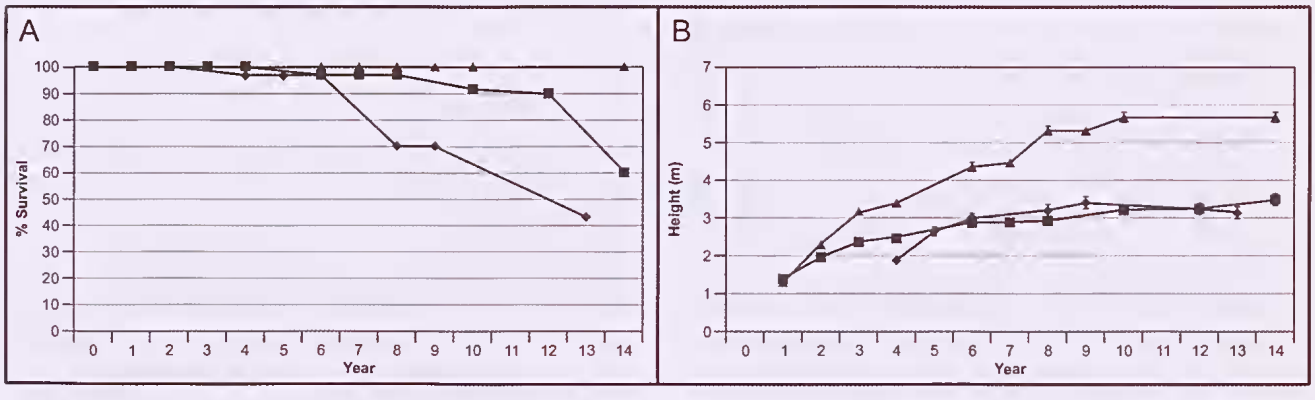

Figure 2. Survival (A) and mean growth (B) of *Melaleuca* at Geraldton ▲, Carnarvon ■ and Kununurra ◆. Bars on height data indicate standard errors where large enough to display.

Melaleuca showed 60% survival but trees were generally unhealthy with many dead twiggy branches and a final height of only 3.5m (Fig. 2). At Kununurra, neem survival was 83% and trees reached 10 m after nine years and then did not increase in height (Fig. 1). *Melaleuca* had 43% survival. It grew to 3m after six years which was its maximum height (Fig. 2).

Sandalwood survival and growth

Final survival of *S. album* K7 at Kununurra was poor at 33% with most deaths occurring at age 4–6 years, compared with 80–90% survival at Geraldton and Carnarvon (Fig. 3). Clone LS gave only 41% survival at Geraldton compared with 60% at Carnarvon, where most losses were at establishment (Fig. 3).

At Geraldton, sandalwood Clones K7 and LS grew at comparable rates with little increase after 10 years (Figs. 3 and 4). The tallest trees were 5.5m tall (K7) and 4.4m (LS). The mean height of LS was greater than that for K7 but the difference was not significant. Trees at Geraldton were significantly shorter than those at the other two

Table 2

Range of sandalwood heights and oil concentration within clones. Height data are for all surviving trees at year 14, oil concentration is for 4-5 of the tallest trees of each clone.

Location	Clone	Height range (m) Minimum	Maximum	n	Oil % concentration range Minimum	Maximum	n
Kununurra	R32*	2.2	7.0	10	0.02	3.85	5
	K7	3.2	8.2	8	2.95	5.71	4
Carnarvon	K7	3.9	9.6	19	0.65	4.03	5
	LS	3.9	9.6	12	2.47	3.55	5
Geraldton	K7	1.4	5.4	14	na	na	
	LS	1.5	4.4	8	na	na	

*on *A. mangium*

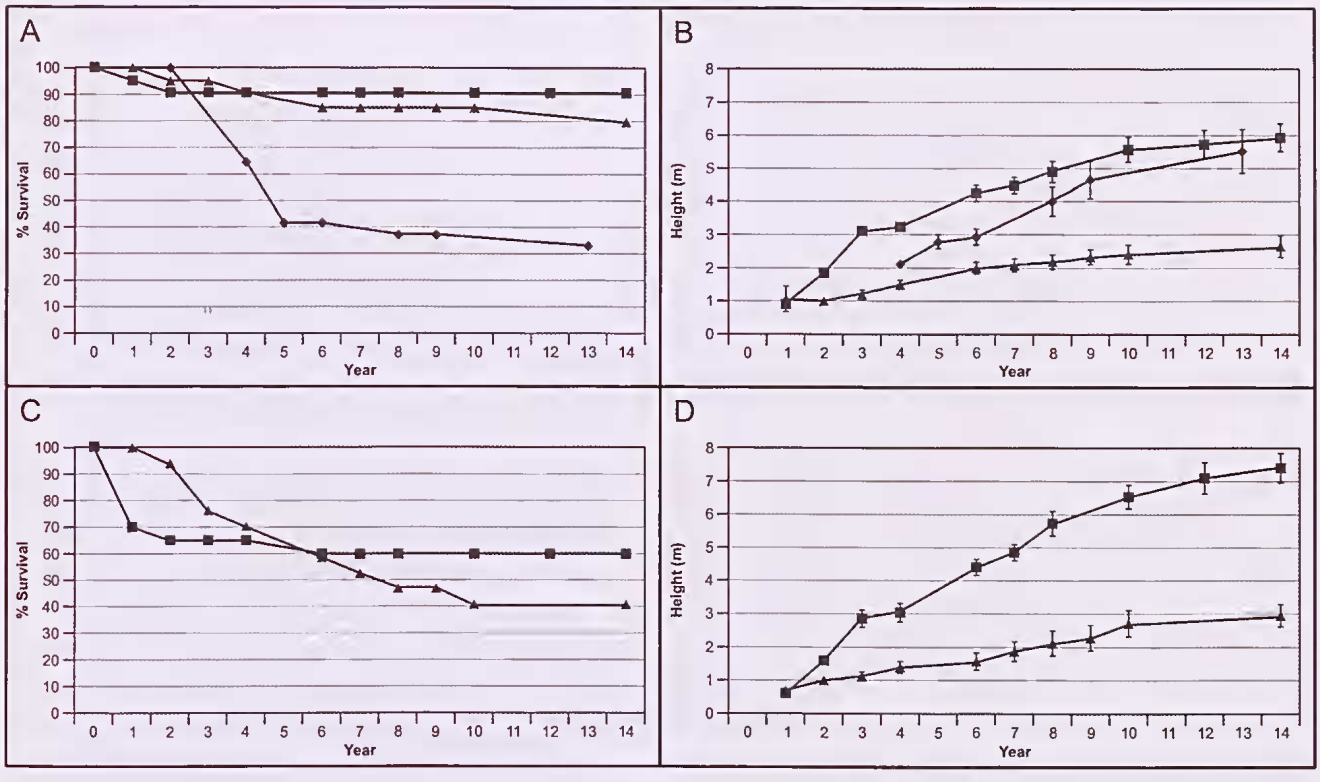

Figure 3. Survival and mean growth of two *S. album* clones K7 (A and B) and LS (C and D) at Geraldton ▲, Carnarvon ■ and Kununurra ◆. Bars on height data indicate standard errors where large enough to display.

Table 3

Sandalwood trunk mean diameters (overbark) at the base and 1.5m height in year 14 for sandalwood clones K7 and LS grown on *Melaleuca* and neem at three locations.

Location	Clone K7 diameter (cm) (SE)			Clone LS diameter (cm) (SE)		
	Base	1.5m	No.	Base	1.5m	No.
Geraldton	6.8 (0.91)	3.5 (0.60)	16	6.6 (1.00)	3.7 (0.70)	7
Carnarvon	16.7 (1.02)	10.9 (0.87)	19	19.3 (1.62)	13.6 (1.22)	12
Kununurra	13.2 (1.05)	10.2 (0.83)	8	na	na	

na: not available

sites. Growth of sandalwood was very similar at Carnarvon and Kununurra with clone K7 growing faster at Carnarvon than Kununurra. At Carnarvon, clone LS had significantly greater mean height than K7 (7.4 compared to 6.0m) (Fig. 4). The range in plant heights of individual clones was considerable (Table 2).

There was little difference between trunk diameters of K7 trees grown at Carnarvon or Kununurra, while those at Geraldton were significantly narrower (Table 3). At Carnarvon, clone LS had greater diameter than K7, but this was not the case at Geraldton. The mean annual increment of diameter at breast height in the trials at Carnarvon and Kununurra was around 8 mm.

Table 4

Mean trunk diameter (overbark) and percentage heartwood for all sandalwood trees from which oil was analysed. SE of means are shown in parenthesis. (All trees had heartwood at the base; only samples with heartwood are included in the data for 1.5m)

Host	Base Trunk diameter (cm)	Heartwood (%)	n	1.5m Trunk diameter (cm)	Heartwood (%)	n
Clone K7 at Kununurra						
Melaleuca & neem	13.9 (0.95)	42.9 (5.1)	7	11.5 (0.91)	30.9 (13.07)	4
Clone K7 at Carnarvon						
Melaleuca & neem	14.7 (1.29)	46.9 (4.49)	9	10.7 (0.74)	29.4 (2.39)	5
Clone LS at Carnarvon						
Melaleuca & neem	18.1 (1.36)	43.7 (4.13)	7	14.1 (1.48)	27.8 (8.90)	5
Clone R32 at Kununurra						
Acacia mangium	13.3 (1.19)	17.5 (5.00)	13	9.2 (0.97)	6.2 (4.98)	4
Dalbergia sissoo	13.0 (0.95)	28.6 (3.00)	15	9.9 (0.83)	3.8 (1.64)	4
Cassia siamia	8.6 (0.33)	34.9 (4.00)	16	6.2 (–)	17.7 (–)	1

Figure 4. A. *S. album* at Carnarvon in year 12. The first tree in the row is a 6m neem, then a K7 sandalwood (8.8m) and an LS sandalwood (9.6m). B. *S. album* at Geraldton in year 15. From left two sandalwood K7 clones (4.4 and 5.4m tall) growing next to *Metaleuca quinquenervia* with an infill *Acacia acuminata* in the foreground.

Sandalwood on three host species at Kununurra

Survival and growth of hosts

C. siamea had almost 100% survival until year nine, but dropped to 56% by year 13 (Fig. 5). Growth plateaued after year nine at 11m. Survival of *A. mangium* and *D. sissoo* fell away steadily until at year 15 there were only 21% of both species alive (Fig. 5).

Survival and growth of sandalwood R32

Only 21–28% of the clone R32 sandalwood on the three different hosts survived with most deaths being over the first four years (Fig. 5). Sandalwood trees reached 4.0 m on *C. siamea*, 5.0 m on *A. mangium*, and 6.1 m on *D. sissoo*. Trunk diameters of trees that were used in the oil analysis, were similar between sandalwood grown with *D. sissoo* and *A. mangium*, both at the base and at 1.5 m, but were significantly smaller on *C. siamea* (Table 4).

Plant form

Clones K7 and LS required light pruning in the first five years to attain a long straight bole. The clone R32 had poor form (almost plagiotropic) and required heavy pruning in years 1–5 to achieve an upright tree. Inadequate weed control within rows meant that many trees developed a trunk bent towards the light in the mown alleyways between the rows). The roots of the excavated trees at both Kununurra and Carnarvon were very shallow and thin (Fig. 6).

Heartwood formation and oil analysis

Effect of site and clone on heartwood (Clones K7 and LS)

Heartwood was first detected in the larger trees at Carnarvon when drilled in year 10. No heartwood was apparent from drilling in Geraldton up to year 14. There was no significant difference between the mean percentage heartwood between clones K7 and LS at Carnarvon, or between K7 when grown at Carnarvon and Kununurra (Table 4). When the oil concentration and composition of K7 grown at Kununurra and Carnarvon were compared, the concentration at Kununurra was much higher from the basal samples, but not at the 1.5m height, and the α- and β-santalol were similar in both locations for samples at the same heights (Table 5). There was a wide range in percentage oil concentration within clones (Table 2). A detailed analysis of the oil constituents is shown in Table 6, with the most important components being α- and β-santalol, bergamotol and epi- β-santalol.

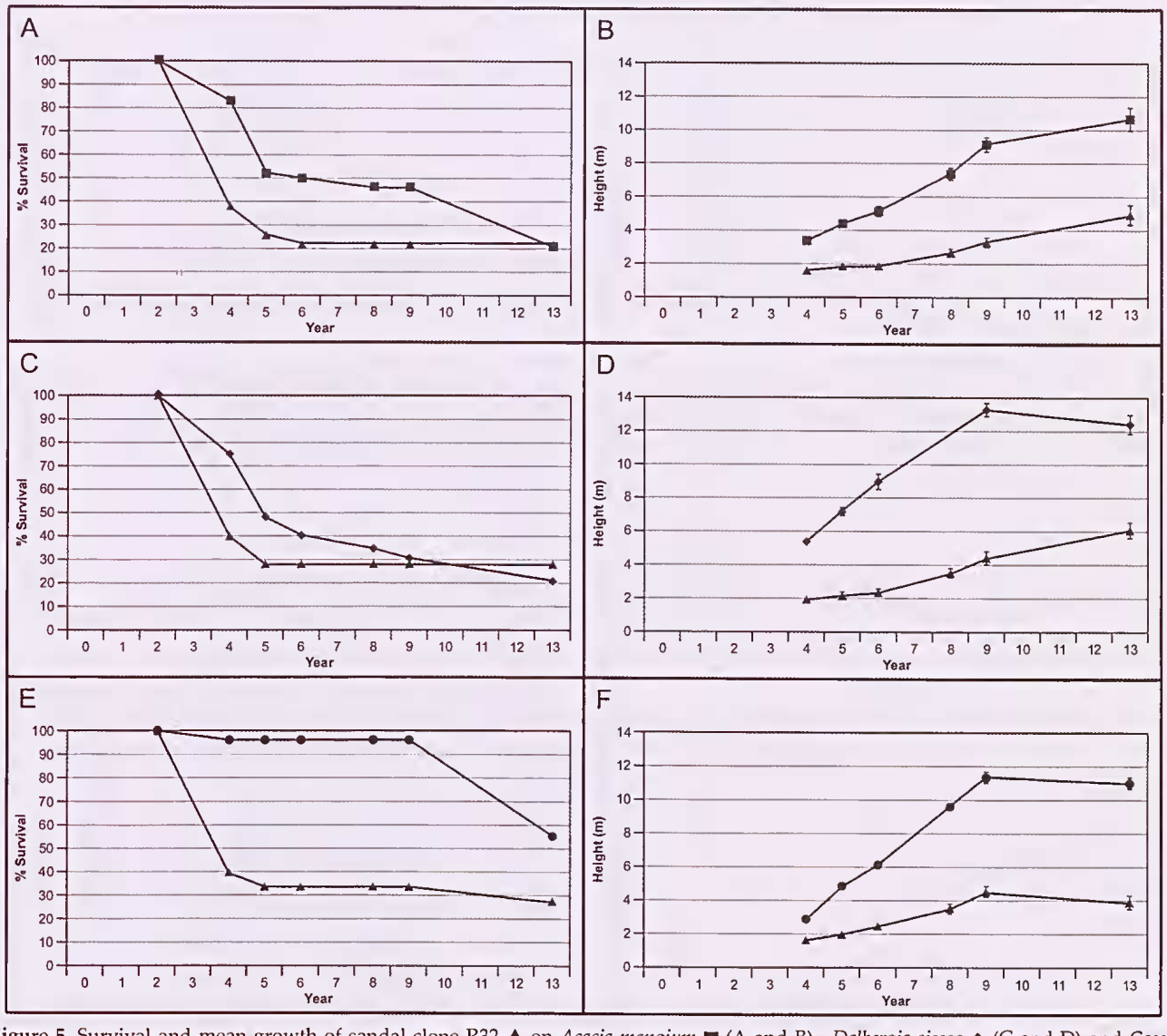

Figure 5. Survival and mean growth of sandal clone R32 ▲ on *Acacia mangium* ■ (A and B), *Dalbergia sissoo* ◆ (C and D) and *Cassia siamea* ● (E and F) at Kununurra. Bars on height data indicate standard errors where large enough to display.

Figure 6. An excavated *S. album* root system at Carnarvon

Table 5

Mean % oil concentration and % α- and β-santalol for clone K7 grown at two localities, Kununurra and Carnarvon.

Location	Oil concentration (%) (SE)	n	α-santalol (%) (SE)	n	β-santalol (%) (SE)	n
Base						
Kununurra	4.38 (0.727)	4	44.05 (1.071)	4	21.91 (0.587)	4
Carnarvon	1.90 (0.590)	5	43.66 (0.703)	5	20.60 (1.138)	5
1.5m						
Kununurra	1.16 (0.653)	4	43.3 (0.704)	2	20.6 (1.139)	2
Carnarvon	1.23 (0.718)	5	41.83 (0.706)	3	19.56 (0.335)	3

A comparison of the two clones growing on the same site (K7 and LS at Carnarvon) showed the two clones having similar concentrations at the base. At 1.5m, there was the same oil concentration in both clones with K7 being slightly higher in β-santalol (Table 7).

Comparison of oil concentration and composition at different heights in the roots and trunk showed only small differences between sections (Table 8). A comparison of clone K7 at Carnarvon and Kununurra indicates that concentration was higher at Kununurra at most positions in the tree but that α-and β-santalol were consistently higher at Carnarvon (Table 9).

Table 6

Mean oil concentration and composition from the base samples of K7 trees from Kununurra and Carnarvon (n=9).

	Mean concentration (g/kg) (SE)	Composition (%) (SE)
α-santalene	0.34 (0.057)	1.26 (0.134)
α-trans-bergamotene	0.06 (0.011)	0.23 (0.27)
epi-β-santalene	0.36 (0.061)	1.32 (0.123)
β-santalene	0.50 (0.083)	1.88 (0.211)
α-santalol	13.18 (2.761)	43.83(0.577)
cis-α-trans-bergamotol	1.85 (0.425)	5.89 (0.266)
epi-β-santalol	1.03 (0.222)	3.41 (0.097)
β-santalol	6.53 (1.445)	21.18 (0.686)
cis-nuciferol, curcumen-12-ol isomer	0.22 (0.069)	0.65 (0.133)
cis-lanceol	0.28 (0.114)	0.74 (0.173)
Total (g/kg)	30.02 (6.126)	80.40 (1.118)
Concentration (%)	3.00 (0.613)	

There was some evidence that faster growing trees had less oil content as the correlation between height growth and oil concentration (%) for LS was (r= -0.73) and for K7 (r= 0.11). The clonal lines showed considerable variation in oil concentration within the same genotype (Table 3).

Table 8

Mean % oil concentration and α- and β-santalol for all clones at different positions from the root to the height where heart wood ceased.

Height (cm)	Oil concentration (%) (SE)	α-santalol (%) (SE)	β-santalol (%) (SE)
–80	0.06 (–)		
–60	2.41 (2.340)	36.05(–)	16.96 (–)
–40	1.81 (1.770)	41.96 (–)	20.92 (–)
–20	1.85 (0.838)	42.64 (2.069)	21.01 (1.072)
0	2.73 (0.870)	44.97 (0.695)	20.76 (1.019)
20	3.64 (1.191)	44.15 (1.149)	21.30 (0.850)
40	2.43 (0.846)	44.38 (0.260)	20.74 (1.297)
60	1.60 (0.490)	42.21 (3.380)	20.34 (0.681)
80	1.68 (0.486)	43.70 (1.572)	20.11 (0.770)
100	2.58 (0.631)	45.18 (0.571)	21.32 (0.429)
120	2.79 (0.697)	44.90 (0.711)	20.77 (0.684)
140	2.47 (0.711)	41.69 (1.566)	19.08 (0.971)
160	1.37 (0.617)	43.50 (1.332)	20.08 (0.517)
180			
200	0.84 (0.643)	39.16 (7.830)	17.74 (4.540)
220			
240	1.08 (0.915)	20.22 (12.203)	9.15 (6.613)
260			
280	1.57 (1.460)	31.03 (11.455)	13.79 (6.610)
300			
320	2.6 (–)	40.36 (–)	20.11 (–)
340			
360	2.41 (–)	38.4 (–)	18.20 (–)
380			
400	2.16 (–)	45.52 (–)	20.22 (–)

Table 7

Mean % oil concentration and α- and β-santalol for clones K7 and LS grown at Carnarvon.

Clone	Oil concentration (%) (SE)	n	α-santalol (%) (SE)	n	β-santalol (%) (SE)	n
Base						
LS	2.88 (0.200)	5	43.04 (2.274)	5	18.97 (0.851)	5
K7	1.90 (0.590)	5	43.66 (0.703)	5	20.60 (1.138)	5
1.5m						
LS	1.23 (0.311)	5	37.98 (2.767)	5	15.40 (1.372)	5
K7	1.54 (0.837)	4	41.83 (0.706)	3	19.56 (0.335)	3

Table 9

Mean % oil concentration and α- and β-santalol for clone K7 trees at Kununurra and Carnarvon at different positions from the root to the height where heart wood ceased (two trees were assessed from each location).

Height (cm)	Oil concentration (%) Carnarvon	Kununurra	α-santalol (%) Carnarvon	Kununurra	β-santalol (%) Carnarvon	Kununurra
–80	0.06 (–)		0.00 (–)		0.00	
–60	0.07 (–)		0.00 (–)		0.00	
–40	0.03 (0)	0.06 (–)	1.72 (1.72)	1.57 (–)	0.00	0.33 (–)
–20	2.39 (0.46)	0.04 (0.02)	41.21 (2.59)	18.38 (0.24)	20.33 (1.43)	9.20 (0)
0	1.07 (0.42)	4.51 (1.20)	44.22 (1.58)	32.53 (1.09)	19.65 (1.75)	15.46 (0.69)
20	1.51 (0.38)	6.50 (0.49)	43.33 (2.80)	31.95 (2.00)	20.50 (1.06)	15.80 (0.86)
40	1.15 (0.30)	4.22 (1.31)	44.26 (0.59)	31.98 (0.40)	19.57 (2.16)	15.65 (0.03)
60	1.20 (0.26)	1.64 (1.38)	45.73 (1.68)	30.72 (8.21)	21.73 (0.60)	15.34 (0.78)
80	0.80 (0.29)	2.17 (1.00)	40.89 (3.25)	31.21 (0.85)	19.39 (1.99)	14.93 (0.22)
100	1.42 (0.44)	4.03 (0.39)	44.33 (0.42)	32.34 (1.30)	21.84 (0.32)	15.87 (0.08)
120	1.39 (0.62)	4.10 (0.87)	45.18 (0.45)	32.32 (1.93)	20.41 (1.98)	15.37 (0.65)
140	1.70 (0.87)	2.58 (1.68)	40.15 (4.40)	30.16 (0.46)	18.62 (2.60)	14.18 (1.52)
160	1.85 (1.60)	1.02 (0.93)	41.31 (0.83)	27.46 (13.62)	19.31 (0.40)	13.04 (6.44)
180						
200	0.38 (–)	0.03 (–)	31.33 (–)	1.07 (–)	13.20 (–)	0.00 (–)
220						
240	0.12 (–)		13.42 (–)	3.32 (–)	4.47 (–)	1.67 (–)
260						
280	0.11 (–)		0.00 (–)		7.18 (–)	

Table 10

Mean % oil concentration and % α- and β-santalol for clone R32 grown on three hosts at Kununurra. In summarising the data on % oil concentration, values of zero were included, but the values for α- and β-santalol were only included for oil concentrations above 0.1%.

	Host *Dalbergia sissoo*	n	*Acacia mangium*	n	*Cassia siamea*	n
Base						
Oil concentration%	0.38 (0.248)	5	2.17 (0.783)	5	0.03 (0.006)	5
α-santalol %	19.99 (6.045)	4	37.06 (2.648)	4	–	0
β-santalol %	9.25 (2.975)	4	17.12 (0.933)	4	–	0
1.5m						
Oil concentration%	0.18 (0.093)	5	0.51 (0.408)	5	0.09 (0.089)	5
α-santalol %	15.36 (8.944)	4	27.44 (6.938)	2	28.07 (0)	1
β-santalol %	7.25 (4.608)	4	13.14 (3.846)	2	13.14 (0)	1

Effect of host on heartwood in Clone R32

At Kununurra, the percentage heartwood was similar in trees grown on *A. mangium* and *D. sissoo* (Table 4). Sandalwood trees growing on *A. mangium* as a host had a higher oil concentration, and α- and β-santalol at both the base and at 1.5 m (Table 10). Sandalwood trees grown on *C siamea,* were smaller than those on *A. mangium* or *D. sissoo.* The oil concentration was negligible at the base of all five trees sampled and only one tree had heartwood at 1.5 m. although this single sample had a high per cent heartwood and similar α-and β-santalol to the trees on *A. mangium* (Table 10).

Discussion

The range of locations used in these clonal trials showed that the warmer, frequently irrigated Carnarvon site and Kununurra with monsoonal rains and winter irrigation were suitable for plantations of *S. album,* but the colder, less frequently watered Geraldton site was not. The annual increment (at breast height) in the trials at Carnarvon and Kununurra was 8 mm pa, which was in the middle of the range reported from India (3–16 mm pa, Venkatesan 1980). The trees in Geraldton averaged only 2.3 mm pa.

Clones did not give any more even growth or oil concentration than would be expected from seedlings but improved silviculture methods might reduce the variability. There were small but significant differences in clone height and diameter with clone LS being superior to clone K7 and possibly clone R32, although this was not grown at the same site. It was necessary to prune all genotypes to achieve an unbranched bole, but clone R32 was initially particularly poor, with thin weak stems, presumably an occasional undesirable effect of cloning through somatic embryogenesis in vitro.

Oil concentration and composition obtained from the 14-year-old trees matched published data from juvenile trees in India (Srinivasan *et al.* 1992). All trees from ramets of clones K7, LS and R32 had heartwood at the base after 14 years when grown at Carnarvon or Kununurra. A higher oil concentration and more valuable composition is expected in heartwood of older trees. The remaining half of the clonal trial at Carnarvon

will provide useful information on oil in trees of different ages.

The 14-year-old trees had less oil concentration in roots compared to trunks, and the roots developed at this age were thin with significant oil concentration only to 20 cm depth (with traces to 80 cm depth). This is contrary to published data for mature Indian trees that showed the lateral roots with high oil concentrations (Srinivasan *et al.* 1992).

Oil content was higher in trees grown at Kununurra compared to Carnarvon, and one of the two clones at Carnarvon had a higher percentage of santalols. Data for 10 year old sandalwood trees grown at Kununurra (Jones *et al.* 2007), cannot be compared to those in this paper as the earlier data included sap as well as heartwood in the analysis.

Dalbergia sissoo, which provided dappled shade and was the tallest of the host species amongst the legumes tested using clone R32, supported the best growth of sandalwood while those hosted onto *A. mangium* showed highest oil concentration. The low survival of hosts was a problem as there were very few host trees of *A. mangium* or *D. sissoo* remaining at year 14. A higher host-to-parasite ratio may be necessary to improve long-term sandalwood survival and growth. It is important to establish permanent hosts in a ratio that will support mature sandalwood at plantation establishment as infill of hosts amongst existing sandalwood was difficult.

Melaleuca and neem were chosen as hosts in this experiment as it was thought that they would survive at all three locations. However, the winter temperatures at Geraldton proved unsuitable for neem. In any case neem is not recommended as a host as in some areas of northern Australia it has become an environmental weed from berries carried by birds to riparian zones (Grice 2002). Nitrogen fixing hosts appear beneficial as indicated by the better growth of sandalwoods at Geraldton that fortuitously attached to nearby wild *Acacia accuminata* and those at the Carnarvon trial next to the street planting of *Casuarina cunninghamiana*. Thus the potential for growth and heartwood production is probably underestimated from the work using *Melaleuca* and neem as hosts, and this work suggests a higher host to sandal ratio might also be beneficial.

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